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Characterisation of mutations in GroES that allow GroEL to function as a single ring

Han Liu [a,b], Eszter Kovács [a,c], Peter A. Lund [a,*]

[a] School of Biosciences, University of Birmingham, Birmingham B15 2TT, UK
[b] The Physiological Laboratory, University of Liverpool, Liverpool L69 3BX, UK
[c] Solvo Biotechnology, H-2040 Budaörs, Gyár u. 2., Hungary

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ABSTRACT

The chaperonin GroEL contains two seven-subunit rings, and allosteric signals between them are required to complete the GroEL reaction cycle. For this reason SR1, a mutant of GroEL that forms only single rings, cannot function as a chaperone. Mutations in SR1 that restore chaperone function weaken its interaction with the cochaperonin GroES. We predicted that GroES mutants with reduced affinity for GroEL would also restore function to SR1. To test this, we mutated residues in GroES in and near its contact site with GroEL. Nearly half of the mutants showed partial function with SR1. Two mutants were confirmed to have reduced affinity for GroEL. Intriguingly, some GroES mutants were able to function with active single ring mutants of GroEL.

1. Introduction

GroEL is the only molecular chaperone which is known to be essential for growth of *Escherichia coli* at all temperatures [1]. GroEL binds a subset of *E. coli* proteins, including several which are essential for cell growth, and allows them to fold under conditions where their aggregation is minimised. The details of this process, including the structural changes undergone by the GroEL protein and the identity of the proteins which require GroEL for their folding, have both been the subject of intense research for a number of years (reviewed recently in Refs. [2–5]). Although the majority of attention has focussed on GroEL, it is only one part of a two-component machine, since the function of GroEL depends on the action of the cochaperonin GroES, which is also essential for growth [1].

GroEL is a double ring complex with seven GroEL protomers in each ring [6]. GroEL sequesters unfolded or partially folded proteins in the cavity at the centre of one of the rings, where they are able to fold without interacting with other unfolded proteins [7–10]. Initially, proteins bind to a hydrophobic patch which is present on the apical domain of each subunit of GroEL, and is hence present as a hydrophobic band around each end of the two rings [11,12]. Binding of ATP to GroEL causes large domain movements, which in some cases partially unfolds the bound substrate, which may assist its subsequent search for the correct folded conformation [2,4,13,14]. Bound proteins are displaced into the central cavity by the binding of the cochaperonin GroES, which caps the cavity for most of the reaction cycle. The bound ATP is then hydrolysed, which weakens the binding between GroEL and GroES, but GroES is not displaced until ATP binds the opposite ring. Loss of GroES uncaps the protein folding cavity, and allows the egress of the substrate, which may bind again to the same or to a different GroEL complex if it has not been completely folded [9,10,15,16].

Structural studies of GroES show that it possesses a flexible loop (generally referred to as the "mobile loop") of amino-acyl residues which is undefined in the free protein but which anchors GroES to GroEL when the two form a complex [17,18]. Three amino-acids at the base of this flexible loop form contacts with the GroEL apical domain, and the formation of these contacts requires a large change in the conformation of the GroEL–ATP complex. This change in conformation leads to the burying of the hydrophobic regions in GroEL, which leads to loss of binding of the substrate protein. Because GroES now caps the GroEL ring, the substrate protein is displaced into the cavity in the GroEL ring, where it remains for the duration of the ATP hydrolysis step, the longest step in the reaction cycle. The protein can thus fold in the cavity without interacting with any other unfolded proteins, the folding possibly being favoured by the limited size of the cavity and the hydrophilicity of the cavity walls [4,5].

The double ring structure of GroEL is needed for completion of the reaction cycle, since information has to pass between the rings

* Corresponding author. Fax: +44 121 414 5925.
E-mail address: p.a.lund@bham.ac.uk (P.A. Lund).

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to signal the progress of the ATP hydrolytic cycle, which takes place on one ring and causes conformational changes in the other [19,20]. Mutants of GroEL which cannot form double rings are incapable of completing the reaction cycle, not because they cannot fold protein, but because the bound GroES cannot be released without an allosteric signal from the other ring [16], thus trapping the folded protein in the GroEL cavity. However, Hsp60 (the mitochondrial homologue of GroEL) has very weak ring–ring interactions but can nevertheless function as a chaperone in vitro and can replace GroEL in *E. coli* as long as it is expressed with its cognate cochaperonin, Hsp10 [21–24]. Moreover, mutations in the inactive single ring form of GroEL can be found which partially restore its function [25,26]. In both cases, the ability of these forms of chaperonin to complete their reaction cycles is due to weakening of their interaction with the cochaperonin, obviating the need for an allosteric signal from the second ring.

The interaction between GroEL and GroES is determined in part by the mobile loop of the cochaperonin [27].

It follows that it should be possible to obtain mutations in GroES that also weaken its binding to GroEL and allow GroEL to function as a single ring, by changing the interaction of the mobile loop with GroEL. In this study, we have explicitly tested this prediction using site-directed mutagenesis of GroES residues at or close to the interaction site, and have tested the properties of these mutants with wild-type GroEL, with an inactive single ring mutant of GroEL (SR1), and with several active single ring mutants. We find that many of these mutations enable the in vivo function of a normally inactive single-ring form of GroEL.

2. Materials and methods

2.1. Strains and plasmids

E. coli K-12 TG1 [28] was used for routine molecular biology procedures. Complementation studies were done in strain MGM100, in which the native promoter of the *groE* operon has been replaced by the tightly glucose-repressible pBAD promoter of the *ara* operon [29]. P1 transduction to delete the chromosomal *groES* and *groEL* genes was from stain NL192Ω [30]. All protein expression for complementation experiments was done from derivatives of the expression plasmid ptrc99A [31]. Protein expression for protein purification was either from the same plasmid, or from pET22b in *E. coli* BL21 (DE3) (Novagen).

2.2. Growth conditions and complementation assays

Bacteria were grown in L-broth or on L agar plates at 37 °C unless otherwise indicated. P1 transductions were done as described in [26]. For complementation experiments, overnight cultures were adjusted to an OD600 of 0.8–0.9 with L-broth, then diluted in L-broth from 10^{-1} to 10^{-6}. Each dilution was spotted onto LB agar plates (containing 0.2% arabinose or glucose, 50 μg/ml kanamycin, 100 μg/ml ampicillin, with or without 0.1 mM IPTG for MGM100 strains). Plates were incubated at the experimental temperature overnight and scored for growth the following day. All assays were done with a minimum of three independent biological replicates. Growth equivalent to wild-type was assigned a value of 4, and for each 10-fold reduction in growth (as judged by colony number and size), this value was reduced by 1. Thus, reduction in growth by 10^4-fold or more was scored as zero growth. To convert these values to "low", "moderate" or "high", values were summed for each mutant under all conditions and expressed as a percentage of the wild-type. Values greater than 67% were recorded as high, 33–66% as moderate, and less than 33% but greater than zero as low.

2.3. Molecular biology methods

Enzymes for DNA manipulation were purchased from New England Biolabs and used according to the manufacturer's instructions. Plasmid preparations were done using either the Qiagen QIAprep Spin Miniprep kit (Catalogue No. 27104) or the Sigma GenElute™ Plasmid Maxi-prep Kit (Product No. PLX15) according to the manufacturer's instructions. Oligonucleotides for site-directed mutagenesis were synthesized with all possible combinations of bases at the appropriate positions, by Alta Biosciencs (University of Birmingham). Site-directed mutagenesis was done using the Quikchange Site-directed Mutagenesis kit (Stratagene) according to the manufacturer's instructions. Mutants were identified by sequencing. DNA sequence analysis was done using the BigDye version 3.1 sequencing kit (Applied Biosystems), the products of sequencing reactions were run by the Functional Genomics Laboratory at the University of Birmingham.

2.4. Protein purification

Wild-type and mutant GroEL was purified as previously described [26]. GroES proteins were his-tagged with six additional histidine residues at their C-terminus, and purified as follows: 20 ml of an overnight culture was diluted into 1 l of fresh medium and grown at 37 °C for 2 h with shaking, and expression was induced (1 mM IPTG, 3 h). Cells were harvested by centrifugation and the pellet was resuspended in 40 ml 5% glycerol/1 mM PMSF, recentrifuged, resuspended in lysis buffer (20 mM NaH_2PO_4, 500 mM NaCl, 20 mM imidazole, pH 7.4, 1 mM PMSF) and lysed by sonication. The supernatant was cleared by centrifugation and passage through a 0.2 μm filter and loaded onto an equilibrated 1 ml Amersham HisTrap FF column by an Amersham AKTA explorer HPLC system. The column was washed with binding buffer (20 mM NaH_2PO_4, 500 mM NaCl, 40 mM imidazole, pH 7.4, 1 ml/min) until the absorbance reached a steady baseline. Proteins were eluted with elution buffer (20 mM NaH_2PO_4, 500 mM NaCl, 500 mM imidazole, pH 7.4 with H_3PO_4) for 5 minutes. Fractions were analysed using 12% SDS–PAGE, pooled, and dialyzed overnight against buffer G (20 mM MOPS, 100 mM KCl, 10 mM $MgCl_2$, pH 7.4). Some GroES mutant proteins were found to precipitate after dialysis, so these were prepared in the presence of 50% glycerol. Dialyzed samples were applied to an equilibrated Amersham Sephacryl S300 column. Proteins were eluted with buffer G2 or G5 (20 mM MOPS, 100 mM KCl, 10 mM $MgCl_2$, 20% or 50% glycerol, pH 7.4). Collected fractions were analysed on 12% SDS–PAGE and pooled as appropriate.

2.5. ATPase assays

The ATPase activity assays were done using EnzCheck phosphate assay kit (Molecular Probes). Reactions were set up according to the manufacturer's instructions except that a different buffer was adopted (10% or 50% glycerol, 25 mM Tris pH7.5, 5 mM KCl, 5 mM $MgCl_2$, and 2 mM DTT). Final concentration for GroES and GroEL proteins in the reaction mix were 0.4 μM and 0.2 μM, respectively (oligomer). ATP concentration was 1 mM. The reaction mix was incubated at 22 °C for 10 min before GroEL was added, after which absorbance at 360 nm was measured continuously for 180 s. ATPase rates were calculated according to a standard curve generated with inorganic phosphate.

3. Results and discussion

The structure of GroES–GroEL–ADP_7 complex [18] shows that the major contacts between GroES and GroEL are mediated by

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three hydrophobic residues in the mobile loop of GroES (I25, V26, and L27), which bind in a hydrophobic groove formed by [redacted] helixes (helixes H and I) in the apical domain of GroEL (Fig. 1a). We aligned GroES homologues from twenty two different bacteria, mitochondria, and chloroplasts, and confirmed that these three residues are highly conserved (Fig. 1b), as is the glycine residue immediately before them (G24 of GroES). These four residues were selected for mutagenesis. Each was mutated to each of the other 19 amino-acids, producing 76 GroES mutants in total. Plasmids were constructed expressing each of these mutants, together with the inactive single ring GroEL mutant SR1, under the control of the ptrc promoter, and these were transformed into MGM100 for complementation analysis. The transformants were tested for growth on plates containing 0.2% glucose or 0.2% arabinose at a range of temperatures from 18 °C to 37 °C, in the presence or absence of 0.1 mM IPTG to modulate levels of expression of the plasmid-borne *groES* and *SR1* genes.

Growth in these experiments could arise in four ways. First, mutations in GroES may arise that allow SR1 to function: these are the mutations of interest. Second, recombination between the plasmid-borne *SR1* gene and the chromosomal *groEL* gene could lead to expression of the wild-type GroEL protein from the plasmid promoter. Third, a mutation might arise in SR1 that independently allows it to function, either as a single ring or by creating an active double ring structure. Finally, a mutation on the chromosome of MGM100 might allow expression of the chromosomal *groE* operon even in the presence of glucose. To rule out the latter three possibilities, the complete sequences of the *groES* and *SR1* genes were determined for all plasmids which showed evidence of enabling restoration of growth of MGM100 on glucose. Plasmids were retransformed into fresh MGM100 to confirm that the phenotype segregated with the plasmid. Extracts from all strains were screened on native gels, which clearly distinguish the single and double ring forms of GroEL [32]. In no cases were examples found of proteins that ran as double rings on native gels (examples are shown in Fig. 2) or of plasmids that contained mutations anywhere other than those inserted by site-directed mutagenesis (data not shown).

Complementation was measured by scoring growth. Liquid culture experiments are challenging with this system as repeated dilutions are needed to deplete GroES and GroEL to a level where they become limiting [21,29,33], so we scored growth on solid media on a scale of 0–4 as described in Section 2. Examples of such plates are shown in Fig. 3. Growth was scored at a range of temperatures, both with and without IPTG induction, and on the basis of these data all mutants were classified as high, moderate, low, or null. These results are summarized in Table 1; full details are given in Supplementary materials. They show that a significant proportion of the GroES mutants enable function of SR1 in vivo to some extent. As the abilities of single ring mutants of GroEL to function in vivo is a direct reflection of their capacity to fold proteins [21,25,26], it can be assumed that these mutations in GroES are allowing SR1 to complete a complete folding cycle.

To rule out the possibility that low residual levels of wild-type GroEL were influencing these results, we deleted the chromosomal *groES* and *groEL* genes from MGM100 using P1 transduction from the strain NL192Ω [30] in the presence of the highly active mutant GroES I25F and SR1. The phenotype of the resulting strain when

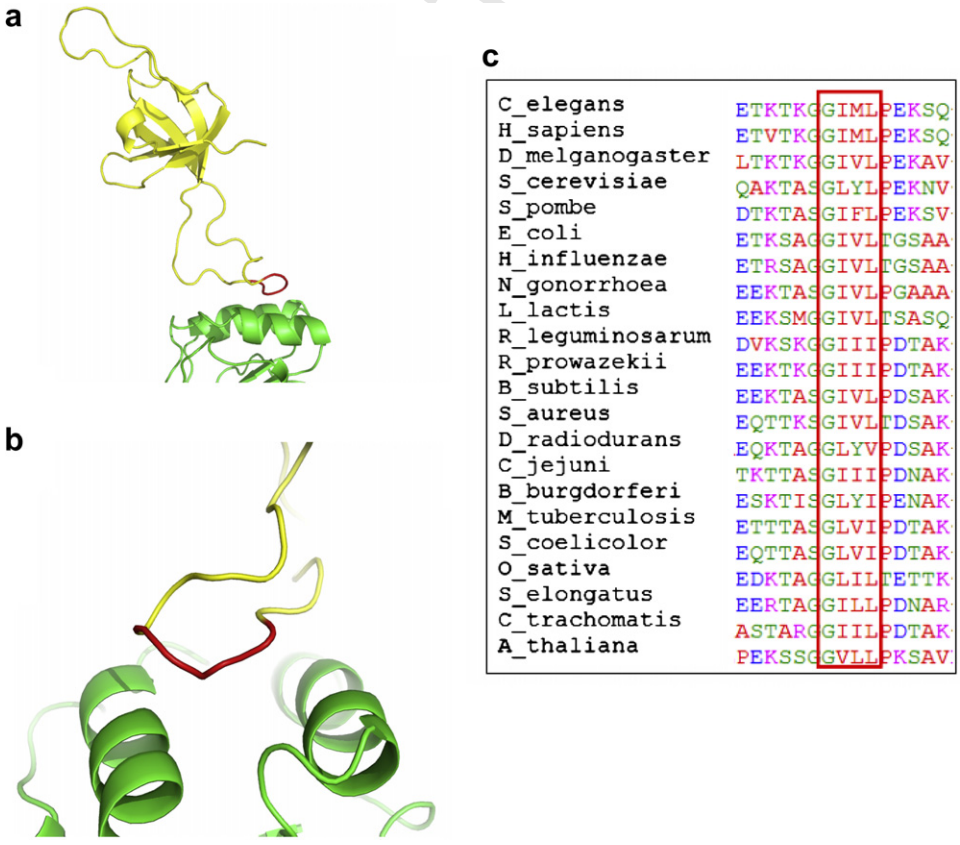

Fig. 1. (a) GroES (yellow) and top of apical domain of GroEL (green), with the four amino-acids of GroES mutated in this study coloured red. The image is of structure 1AON from the Protein Databank, viewed using Pymol [37]. (b) Same structure and colour scheme as (a) but rotated and magnified to show the interaction of the bottom of the GroES mobile loop with the two helices at the top of the GroEL apical domain (c) ClustalW alignment of the tip of the GroES mobile loop and flanking amino-acids of twenty two GroES homologues from bacteria and eukaryotes (five mitochondrial, two chloroplast, 15 bacterial). The four amino-acids targeted for mutagenesis are boxed in red.

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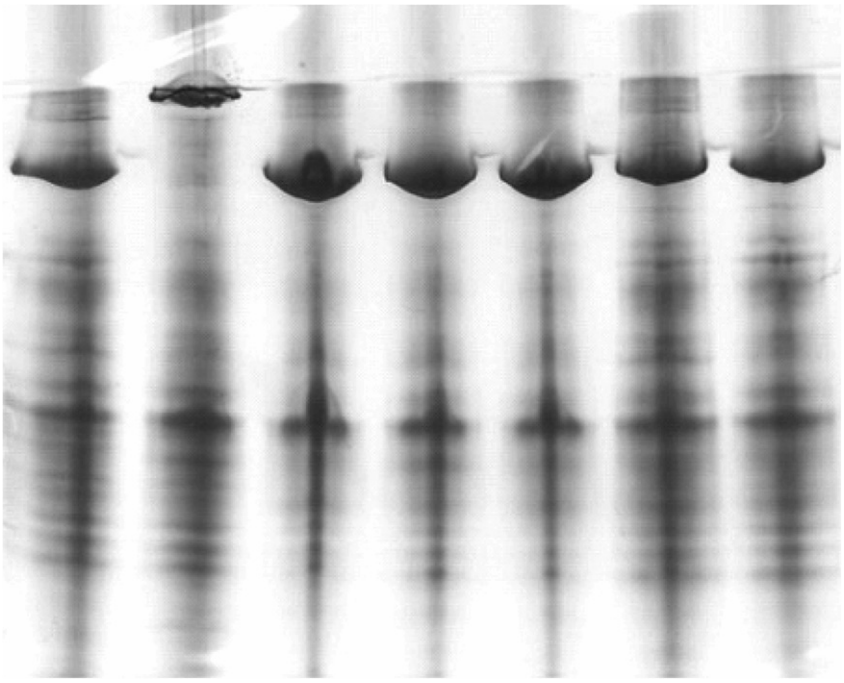

Fig. 2. Native gel electrophoresis of extracts from MGM100 cells grown in LB + 0.2% glucose, 0.1 mM IPTG, expressing the following combinations of GroES and GroEL proteins from the ptrc promoter. 1: wtGroES, SR-A92T; 2: wtGroES, wtGroEL; 3: GroES-G24W, SR1; 4: GroES I25F, SR1; 5: GroES I25M, SR1; 6: GroES [illegible], SR1; 7: GroES L27C, SR1. The GroEL and SR1 proteins are indicated by double and single filled arrowheads, respectively, and the GroES bands by a single open arrowhead. (The identity of these proteins was confirmed in separate experiments by immunoblotting; not shown.)

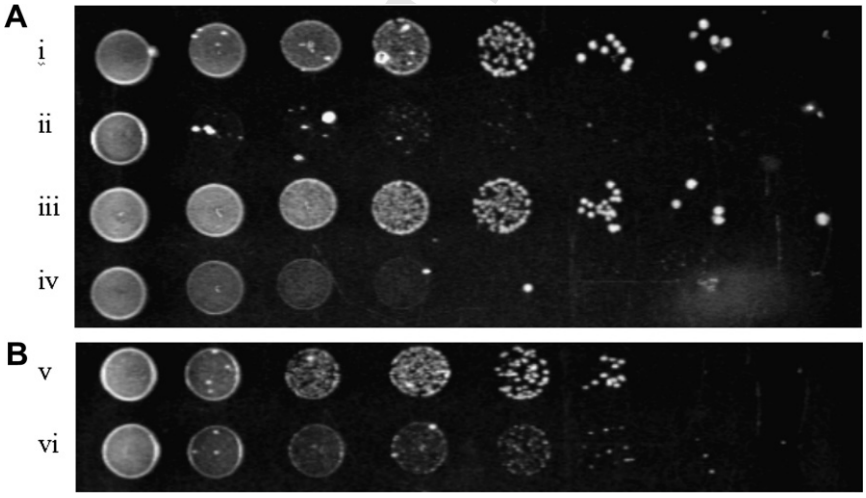

Fig. 3. Examples of complementation plates. These illustrate the scoring system used. All strains are MGM100 expressing combinations of SR1 or GroEL and wild-type or mutant GroES under the control of ptrc grown on LB/0.2% glucose/0.1 mM IPTG. Spots are serial 10-fold dilutions from left to right. Plate A was incubated at 30 °C, plate B at 18 °C. (A) (i) GroES + GroEL (scored as 4); (ii) SR1 + GroES I25L (scored as 0); (iii) SR1 + GroES I25M (scored as 4); (iv) SR1 + GroES I25V (scored as 1). (B) (v) SR1 + GroES L27I (scored as 3); (vi) SR1 + GroES L27M (scored as 2).

Table 1
Functionality of all 76 possible mutations in the four targeted amino-acids of GroES, when co-expressed with the inactive GroEL single ring mutant SR1 in strain MGM100 on LB agar plates with 0.2% glucose, which completely suppresses expression of the chromosomal *groES* and *groEL* genes [29], with or without 0.1 mM IPTG. Growth was scored at 18 °C, 22 °C, 26 °C, 30 °C, and 37 °C, relative to growth under permissive conditions (on 0.2% arabinose). On the basis of growth under all conditions mutants were scored for complementation as null, low, moderate, or high. Full results and an explanation of how phenotypes were scored can be found in the legend to Supplementary materials, Table 1.

Degree of complementation	Position of mutation			
	G24 to	I25 to	V26 to	L27 to
Null	I	A C D E G H K N P Q R S T Y	A C D E F G H I L M P Q W Y	A D E F G K N P S
Low	A E K L M Q R S T V	V	K R S T	I M R V
Moderate	D F H N P Y	L M W	N	H Q T Y
High	C W	F		W C

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grown on glucose was identical to that of MGM100 carrying the same plasmid, confirming that the assay in MGM100 is reliable (data not shown).

Variation in complementation ability between different mutants could be caused by different levels of expression or folding of the different mutant proteins, as well as by different degrees of function of those proteins. We therefore compared levels of expression of all the different mutant forms of GroES on native protein gels, on which GroES multimers resolve clearly from other proteins. No significant variation in levels of the complementing GroES proteins was seen (examples are shown in Fig. 2) except for some cases where no inducible expression could be seen; all of these were non-complementing in our experiments as expected.

We conclude that a substantial proportion of GroES proteins which are mutated in or near to the GroEL-binding region can show partial restoration of chaperonin function when expressed with a normally non-functional single ring GroEL protein. In no case was complete restoration of function seen: all the strains examined grew less well than the positive control. These data are consistent with a model where weakened binding of GroES to GroEL is responsible for allowing the activation of SR1. Studies with purified protein support this model (see below). Given that large numbers of mutations in *groEL* can also easily be obtained that can function well as single rings (27 and PAL unpublished obs.), it is clearly the case that the double ring structure of GroEL is a refinement to give it maximum activity, rather than an absolute pre-requisite for function. Neither the activating mutations in GroES nor any of the mutations that we have obtained in SR1 permit growth at 43 °C. In this context it is interesting to note that it has been proposed that GroEL may, at high temperatures, have more of a protein storage than a protein refolding role, caused by a loss of interaction between the two rings (which would hence prevent the release of a bound peptide from a GroES-capped cavity, as this normally requires an allosteric signal from the *trans* ring) [34,35]. Mutants which destabilized the GroEL–GroES interaction would not be able to function in this way, as GroES would still be able to dissociate.

In addition, the observation that 18 of the 19 possible mutations of G24 allow activation of SR1 confirms that this residue has an important role in the interaction between GroES and GroEL, as suggested by its very high conservation, even though it does not make a direct connection with GroEL. It is likely that the conformational flexibility allowed by this residue is important in allowing optimal binding of the next three residues to GroEL. The fact that even very conservative changes at position I25 allow good activation of SR1, whereas similarly conservative changes at V26 and L27 lead to weak or no activation, shows that the identity of the amino-acid at position 25 is particularly critical in determining the strength of the interaction between GroES and GroEL. Consistent with this, more substantial changes in amino-acid are needed at positions V26 and L27 (for example, V to N, and L to W, C, Q or Y) to have the same phenotypic effect.

3.1. Co-expression of mutant GroES proteins with wild-type and active single-ring mutants of GroEL

We propose that these GroES mutants restore SR1 function by weakening the interaction between GroES and SR1, thus reducing the requirement for an allosteric signal from the absent *trans* ring of GroEL for GroES release. This raises the question of how well these mutants will function with wild-type GroEL, and with the active single ring mutants of GroEL that we have described previously [26]. We addressed this question by co-expressing several different GroES mutants with wild-type GroEL and with four active single ring mutants of GroEL, namely SR-A92T, SR-D115N, SR-A399T, or SR-T522I. The GroES mutants chosen were all ones that gave good growth with SR1. The results of these experiments are summarized in Table 2. All of the GroES proteins that allow SR1 to function also function well with wild-type GroEL, although they did not function as well as wild-type GroES when tested at 42 °C (see Supplementary Table 2). However, we observed that many of the GroES mutants that can function with SR1 also function well with many of the active single ring mutants of GroEL. This was unexpected, since we had predicted that the combined effect of two independent mutations, both of which weaken binding, would significantly reduce or completely abolish chaperonin function. A possible interpretation of this result is that the two sets of activating mutation (i.e. those in GroES and those in SR1) affect different steps in the sequence of events that take place as GroEL goes through the protein folding cycle, and so the effects are not additive. A more detailed analysis of the reaction pathway using purified components will be required to see whether this interpretation is correct.

Relatively minor substitutions at I25 (to L, M, or F) led to mutants with good activation of SR1, whereas at V26 and L27 similar conservative changes had little or no activity with SR1. This suggests that minor changes at V26 and L27 have little effect on the binding of GroES to GroEL, and hence such mutants should still function well with GroEL. This prediction was confirmed for three mutants (V26I, V26L, and L27F; data not shown).

3.2. Purification and properties of selected GroES mutants

Because of the unexpected result with active single ring mutants, we further tested the hypothesis that the activating effect of the GroES mutants described above does indeed come from reduced binding to GroEL. This was done by assessing the effect of purified GroES proteins on the ATPase activity of GroEL. GroES reduces the ATPase activity of GroEL due to inhibition of ADP release [15], and decreasing the strength of the interaction of GroEL and GroES reduces this inhibitory effect, which can thus be used as an indirect measurement of the affinity of GroES for GroEL which correlates well with binding affinity [23,25,26,36].

To select which GroES mutants to purify, we considered the different types of mutant protein that might arise. Some (“tight binders”) would still bind GroEL with close to wild-type affinities: these would still be active with GroEL but not with SR1 in a protein fold-

Table 2
GroES and GroEL (wild-type or mutated) were co-expressed from the ptrc promoter in plasmid ptrc99A in strain MGM100, at 37 °C or 42 °C with or without added 0.1 mM IPTG. Complementation was scored as high, moderate, low or null as described in Supplementary Fig. 1.

Expressed with	GroES mutant												
	wt	G24W	I25F	I25L	I25M	125V	V26K	V26N	V26R	V26S	V26T	L27C	L27T
GroEL	High	Mod	High	High	Mod	Mod	Mod	High	High	High	High	High	Mod
SR-A92T	Mod	Mod	Mod	High	Low	Mod	Mod	Low	Mod	Low	High	Mod	Low
SR-D115N	Mod	Mod	Mod	High	Mod	Mod	Mod	Mod	Mod	Mod	High	Mod	Mod
SR-A399T	Mod	Mod	Mod	High	Null	Mod	Mod	Low	Mod	Low	Mod	Mod	Low
SR-T522I	Mod	Mod	Mod	High	Low	Mod	Mod	Low	Mod	Mod	Mod	Mod	Low

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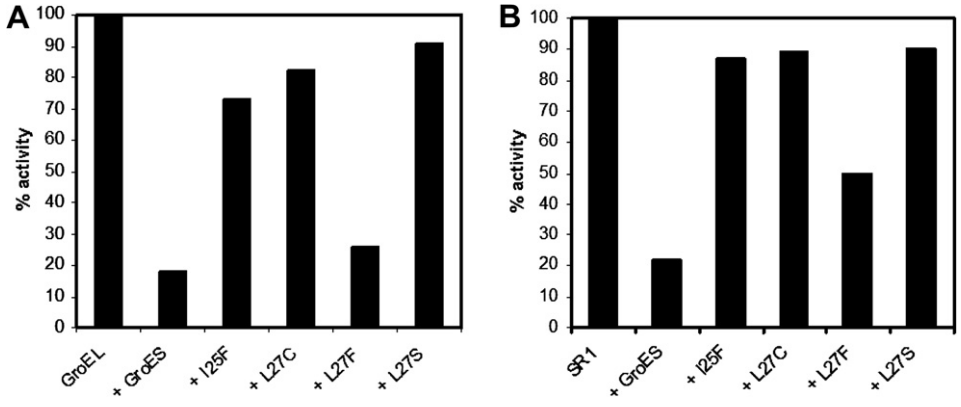

Fig. 4. All four mutant proteins, and wild-type GroES, were his-tagged by cloning the appropriate genes into the vector pET22b-GroES (F.U. Hartl, pers. comm.). We confirmed that the addition of the his-tag had no effect on their in vivo phenotypes (not shown). Proteins were expressed in BL21 and purified to homogeneity in two steps: binding and elution from an Amersham Histrap FF column, and size exclusion chromatography on a Sephacryl S300 column. Analysis on a Superose 6 confirmed that all mutant proteins eluted with the same molecular mass as the wild-type protein. Three of the mutants (all the ones at position 27) tended to aggregate and required the presence of 50% glycerol to be kept in solution, which was therefore used for all ATPase assays. ATPase assays were done as described in Ref. [27], with a 2:1 ratio of GroES to GroEL or SR1, with GroES at 0.4 μM and GroEL or SR1 at 0.2 μM (oligomer). Assays were repeated a minimum of three times; results varied by no more than 10% between replicates.

ing reaction. Mutants which showed some reduced binding ("moderate binders") would be predicted to be active with both SR1 and GroEL. Further reduction of binding ("weak binders"), would produce GroES proteins that would not function with either GroEL or SR1.

GroES I25F and GroES L27C both function well with SR1, and these were chosen for purification as candidate moderate binders. We also purified L27F which functions well with GroEL but not at all with SR1 and is hence a candidate "tight binder", and GroES L27S, which has no activity with either GroEL or SR1 and is therefore a candidate "weak binder".

The effects of these four proteins plus wtGroES on GroEL and SR1 ATPase activities are shown in Fig. 4. wtGroES strongly inhibits the ATPase activity of both proteins, as expected. The effects of L27F (predicted to be a "tight binder") on the ATPase activity of GroEL and SR1 were predicted to be the same as those of wtGroES, and this is indeed the case. Both GroES I25F and GroES L27C, predicted to be "moderate binders", show some reduction in the degree to which they inhibit GroEL and SR1 ATPase, but not as much as wtGroES or GroES L27F, again as predicted. Finally, GroES L27S shows some reduction of ATPase activity of both GroEL and SR1, but in the case of GroEL this reduction is even less than that seen with the "moderate binders". A complication with the assay of GroES L27S is that it is likely to contain some wtGroES in the complex as cells expressing this protein alone cannot be grown; this may explain why there is still some residual reduction of GroEL and SR1 ATPase activity with this protein.

In conclusion, we have shown that reduction of affinity of GroES for GroEL by mutation of the conserved residues at the tip of the GroES mobile loop can enable partial activation of the normally non-functional single ring protein SR1, as judged by regain of in vivo function. A surprisingly high frequency of mutations showed some degree of activation, indicative of a degree of flexibility in the GroEL–GroES system with respect to the requirement for double rings. However, no mutants gave a complete restoration of activity, and in other experiments not reported here using random mutagenesis we have failed to find any fully functional mutants, either of GroES or in SR1, that can do this (data not shown). The double ring of GroEL therefore appears to be required for optimal function at higher temperatures of growth. The in vitro properties of selected GroES mutants correlated well with their in vivo phenotypes with SR1. Interestingly, many of the GroES mutants were still able to function with variants of SR1 that had been selected for their ability to act as single rings. As both types of mutation reduce the GroEL–GroES interaction, we hypothesize that they affect the interaction at different stages during the reaction cycle.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.06.027.

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